REMARKS

Upon entry of the present amendment, claims 6-12 will remain pending in the above-identified application and stand ready for further action on the merits.

The amendments made herein to the claims do not incorporate new matter into the application as originally filed. In support of this contention, the following is noted.

First, claim 1 has been cancelled and its limitations are now recited in claim 6, which is also now presented in an independent format. Claim 6 (unlike prior claim 1) recites a "cured sustained-release formulation" instead of a "solid formulation", finding support at page 1 and Examples 1-2 of the application as originally filed (*see pages 16-18*). Claim 6 also recites the phrase "reacts" instead of "is reacted" as was previously recited in claim 1. Claim 7 has also been amended to change the term "is reacted" to "reacts."

Claim 6 (and claim 7) also recite the phrase *"wherein the active ingredient is either slightly soluble or insoluble in water and the release rate of the active ingredient from the formulation is accelerated in a body fluid"*, which finds full support at page 5, lines 17-20 and page 9, lines 24-26 of the originally filed specification.

Claims 7-11 have also been amended to also refer to a "sustained-release formulation" and the dependency of claims 8 and 10 has been changed based on the cancellation of claim 1 herein. Prior claim 8 is now also broken into two parts (now claims 8 and 12) to prevent a certain degree of redundancy with instantly amended claim 6. As such, new claim 12 finds support in claim 8 as previously amended and at page 10, lines 8-15 of the specification as originally filed.

Entry of the instant amendment is respectfully requested as it does not incorporate new matter into the application as originally filed, and at the same time, puts the pending claims into proper form for issuance in a United States Patent.

Interview With Examiner

On August 24, 2005, the undersigned conducted an Interview with Examiner Tran at the USPTO. The Examiner's courtesy in granting the interview is greatly appreciated. The Examiner's statement in the Examiner Interview Summary Form is correct with regard to subject matter discussed in the Interview.

It is noted that the amendments made herein to the claims have been made in part based on comments made by the Examiner in the Interview in an effort to expedite prosecution of the instant application and claims to allowance.

Claim Rejections – 35 USC § 112

Claims 1, 6 and 8-11 have been rejected under the provisions of 35 USC § 112, first paragraph as failing to comply with the written description requirement. Reconsideration and withdraw of this rejection is respectfully requested based on the following considerations.

In the outstanding Office Action, the Examiner asserts as follows:

> *"...it appears that nowhere in the specification disclose the limitation "the solid formulation" as recited in claim 1."*

This rejection is submitted to be rendered moot as the result of the amendments made herein to the claims, including the cancellation of claim 2 and conversion of claim 6 into an independent claim. In claim 6, the term "cured" not "solid" is used. Support for the term "cured sustained-release formulation" is found at page 15, lines 18-21, page 16, line 9, and in Examples 1 and 2.

Claim Rejections Under 35 USC § 103

Claims 1 and 7-9 have been rejected under 35 USC § 103(a) as being unpatentable over Dunn et al. (US 5,324,519). Further, claims 1 and 6-11 have been rejected under 35 USC § 103(a) as being unpatentable over Fujioka et al. (US 4,985,253) and Dunn et al. (US 5,324,519). Reconsideration and withdrawal of each of these rejections is respectfully requested based upon the following considerations.

Dunn et al. (US 5,324,519)

The Examiner asserts at page 5, lines 5-7 of the Official Action ("OA"):

> *"Contrary to the applicants argument,...it appears that applicant's specification does not disclose a "solid composition" itself".*

This rejection of the Examiner has been rendered moot as a result of the amendment herein to cancel claim 1.

The Examiner also asserts at page 5, lines 7-11 of the Office Action:

> *"It is noted that Dunn teaches the particles that maintain solid form as desired by the applicant. See column 4, lines 58-63, wherein upon contact with an aqueous fluid and the solvent, the thermoplastic polymers are capable of coagulating or solidifying to form a solid matrix suitable for use as an implant. Furthermore, Dunn teaches that the formulation in situ forms a solid...."*

This rejection is submitted to be irrelevant to the present invention. The Examiner seems to confuse the terms "composition (liquid)" and "matrix (solid)" used in Dunn, for example, the Examiner asserts:

> *"Thus, it would have been obvious for one of ordinary skill in the art to modify the biodegradable polymer composition of Dunn to include the carbon in the matrix..." (See page 3, lines 22-25 of the office action).*

Accordingly, Applicants would like to clarify the subject matter of Dunn's invention and show the difference between it and the subject matter of the present invention.

The composition of Dunn is:

> *"A composition suitable for forming an in situ solid implant...the composition being capable of coagulating or solidifying to form a solid or gelatinous microporous matrix upon its contact with aqueous or body fluid, the matrix being a core surrounded by a skin, the core containing pores...(claim 1)."*

Thus, the subject matter "composition" of claim 1 of Dunn is liquid (being capable of coagulating or solidifying to form a solid). It is also clear from the Dunn description that "the composition is a liquid formulation" (see column 2, line 11, of Dunn).

On the other hand, the "matrix" is a solid, which the liquid composition is intended to form after administration. The Dunn description teaches that the matrix is a solid material formed from the composition, for example:

> *"These and other goals are achieved by the present invention which is directed to a composition for providing in situ a biodegradable or bioerodible microporous matrix." (See, column 2, lines 5-8.)*

> *"Another object is to provide a composition which may be administered to an implant site in liquid form and which is capable of solidifying in situ to form an implant." (See, column 1, lines 56-59.)*

Thus, the terms "composition" and "matrix" in Dunn represent quite different states in different stages.

As mentioned above, the Examiner's assertion "to modify the biodegradable polymer composition of Dunn to include the carbon in the matrix" is groundless because the composition represents a liquid state before administration, while the matrix represents a solid state after administration. The Examiner takes these two states, which are different in terms of time and physical structure, for one state of simultaneous coexistence. Therefore, even if a vaccine is

included into the liquid polymer composition of Dunn, it is not the "cured formulation" of the present invention as recited in instant claim 6.

Further, the "matrix" of Dunn is distinct from the "sustained-release formulation" of the present invention for the following reasons. That is, the "matrix" or "solid implant" described at column 4, lines 58-63 and column 4, lines 14-15, respectively, of Dunn to which the Examiner refers (see, page 5, lines 7-14 of the Office Action) is not a formulation, but is instead an intended product to be formed *in situ* from the "liquid composition" of Dunn after administration. On the other hand, the "matrix" is a material having pores that have been formed by carbon dioxide generated from the said liquid composition, and therefore, the "matrix" does not have any direct relationship with an accelerated drug release by carbonate. Instead, Dunn uses carbonate or solvent only as a means for forming pores in the resulting matrix. For example, in Dunn the following descriptions are provided:

- "organic solvent will produce pores" (column 2, line 64);
- "a pore-forming moiety such as carbon dioxide" (column 2, lines 66-67); and
- "the action of the solvent (or pore-forming moiety)...generates pores in the matrix" (column 3, lines 17-20; *emphasis added*).

It is evident that carbon dioxide gas produced from carbonate in Dunn's composition cannot participate in the release of an active ingredient from a solid material, since said gas only participates in the formation of pores when the liquid composition is being solidified into a matrix.

The Examiner's assertion at page 5, lines 11-15 of the Office Action is related to the advantages of the liquid formulation of Dunn's invention and is irrelevant to the cured sustained-release formulation of the present invention. Dunn rather teaches away from the present invention by stating:

"Advantageously, the composition of the invention is useful in overcoming placement difficulties inherent with solid forms of implants" (see column 4, lines 15-18).

It is submitted that one of ordinary skill in the art can understand the technical idea underlying the liquid composition (formulation) as the subject matter of Dunn's invention, and would never dare to turn back to a solid formulation (the disadvantages of which are stressed by Dunn). Therefore, the cured sustained-release formulation of the present invention, which is a typical solid formulation, is actually taught away from by Dunn. That is, even from a technical viewpoint, a person skilled in the art is taught away by Dunn from modifying the liquid composition of Dunn into a cured formulation. That is, the contact with environmental water is significantly low in the case of a cured formulation as compared to a liquid formulation that is miscible with water, and as contact with water is indispensable for pore-formation (carbon-dioxide-generation), such a solid formulation would be very disadvantageous for the purpose of Dunn's invention. The purpose of Dunn's invention is to provide a matrix (solid) with pores that are formed *in situ*. Thus, the cured formulation of the present invention is clearly taught away from by Dunn.

Consequently, the present invention is not obvious from Dunn.

Fujioka et al. (US 4,985,253)

The Examiner asserts:

"Fujioka is silent as to the teaching of vaccine as the active agent." (See, page 4, line 10 of the Office Action).

Further, the Examiner asserts:

"Dunn is cited in combination with Fujioka solely for the teaching of vaccine as an active agent." (See, page 6, lines 8-9 of the Office Action).

Considering these descriptions, the Examiner appears to be incorrectly taking the position that Fujioka's composition contains carbonate. Actually, Fujioka does not disclose or suggest the use of particles containing carbonate even at column 4, lines 40-60 to which the Examiner refers. As for the accelerated release of active ingredients that is achieved by allowing carbon dioxide to generate, which is one of the effects of the present invention (e.g., see claim 6), Fujioka does not disclose or even suggest such an effect.

As such, one of ordinary skill in the art would realize that the present invention is not obvious over Fujioka in combination with Dunn.

In addition, the Examiner has objected to the Applicants' argument in the previous response as follows:

> *"Applicant argues that the combination of Dunn and Fujioka is not an appropriate combination to deny the unobviousness, because...in response to applicant's argument, the test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art."*

The above statement of the Examiner seems to imply that the Applicant has asserted that features of the secondary reference are not bodily incorporated into the structure of primary references (and hence the rejection is unjustified). If so, the Examiner's understanding is incorrect.

The Applicants' previous argument was focused on there being no reason to combine the invention of Fujioka with that of Dunn, because these inventions are utterly different from each other in terms of the problem to be solved and the mean for solving the same. That is, Fujioka is related to a solid composition by which an intended "sustained release" is achieved, whereas Dunn is related to a liquid composition which solves a problem (difficulty in administration) of a solid composition (see column 4, lines 15-18 of Dunn). Furthermore, as described above when discussing Dunn, one

of ordinary skill in the art would not be motivated to try to turn back to a solid formulation of which disadvantages are stressed by Dunn at column 4, lines 15-18. Accordingly, the disclosure of Dunn actually teaches away from combining its teachings with those of Fujioka.

Unexpected Effect of the Present Invention

As the Examiner has pointed out, the reference formulation 2 of Test Example 1 of the present application is not exactly the same as Fujioka's formulation. The difference between reference formulation 2 and that of Fujioka is that glycine is used in the former while human serum albumin (HSA) is used in the latter. As is hereinafter discussed, glycine has an extremely high effect of increasing the release of a drug compared to albumin, and hence the reference formulation 2 of Test Example 1 is a model which is much more liable to release a drug than Fujioka's composition. This means that the comparative experiment of Test Example 1 has been conducted under conditions considerably disadvantageous to the present invention as compared to the Examiner's proposed side-by-side comparison.

The fact that glycine has a higher effect of increasing the release when compared to HSA is supported by **Exhibit 1** (Journal of controlled release 73 (2001) 279-291, especially, at page 284, Fig. 4). Fig. 4 of **Exhibit 1** shows that IFN was not released from a formulation comprising 30% HSA (C-13, cf., Table 1); however, the release rate of IFN was increased (about 10%, on day 30) when 10% of glycine was added to the formulation (C-10, cf., Table 1).

Thus, test Example 1 shows that the present formulation released latex beads at a rate of more than 300-fold compared to the reference formulation 2. Although the reference formulation 2 can release a drug at a much higher rate than Fujioka's formulation, it could release latex beads only at a very low rate of 0.1 ± 0.0 μg/ml.

These facts indicate that the effect of accelerating the release of active ingredients of the present formulation cannot be expected from Fujioka. Likewise, Dunn does not disclose nor even suggest such an effect, and hence the present invention is not obvious from Dunn alone or the combination of Fujioka and Dunn.

CONCLUSION

Based upon the amendments and remarks presented here, the Examiner is respectfully requested to issue a Notice of Allowance clearly indicating that each of pending claims 6-12 are allowable at present.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact John W. Bailey (Reg. No. 32,881) at the telephone number below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Dated: September 13, 2005

Respectfully submitted,

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JWB:jwb:enm
0020-4976P

Attachment: **Exhibit 1** (Journal of controlled release 73 (2001) 279-291)

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Development of a new drug delivery system for protein drugs using silicone (II)

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Received 8 December 2000; accepted 14 March 2001

Abstract

In order to achieve a zero-order release of protein drugs, we have developed a new drug delivery system using silicone, which is named the covered-rod-type formulation. Preparation of the covered-rod-type formulation was conducted under mild conditions without heat treatment or the use of organic solvents. The covered-rod-type formulation released human serum albumin (HSA) or interferon (IFN) at a constant rate for 30–100 days in vitro without significant initial burst. When the IFN covered-rod-type formulation was implanted in nude mice, the serum IFN concentration was maintained at a constant level during the period of observation, i.e., 28 days. The covered-rod-type formulation enabled precise control of the release of the protein drugs and would be expected to increase the duration of the drug effect and to reduce the frequency of administration and side effects. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Zero-order; Covered-rod; Interferon; Silicone; Release mechanism

1. Introduction

The purpose of sustained-release formulations is to maintain an appropriate drug concentration in the body over a long period, therefore, in general, a formulation which releases drugs at a constant rate is considered ideal. However, unlike lipophilic drugs, which can be released at a constant rate from hydrophobic polymer, the number of reports on the successful zero-order release of protein drugs is limited [1,2].

To devise a practical sustained-release formulation for protein drugs, we have studied silicone as a drug carrier [3]. We used interferon (IFN) as an example of a protein drug and produced a matrix-type formulation in which IFN/human serum albumin (HSA) powder is uniformly dispersed in a silicone matrix; we reported that the IFN release rate can be controlled by changing parameters such as the amount of additive and particle size of the IFN/HSA powder. However, the release of protein drugs from a matrix-type formulation is a first-order release, i.e., the release rate decreases with time. We investigated the

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0168-3659/01/$ – see front matter
PII: S0168-3659(01)00302-9

release mechanism of protein drugs from the matrix-type formulation and succeeded in developing a new drug delivery system that enables a constant release of protein drugs over a long period, by improving the geometry of the formulation. In this paper, a technology to control the release of protein drugs from the new silicone formulation, which is named the covered-rod-type formulation, is reported.

2. Experimental

2.1. Materials

Silicone elastomer (Silastic® Q7-4750) was obtained from Dow Corning (MI, USA). Human lymphoblastoid interferon, a natural α-type interferon (IFN) (Sumitomo Pharmaceuticals, Osaka, Japan), was used. Human serum albumin (HSA) (Buminate 25%, Baxter Healthcare) was used to control IFN release from silicone. Glycine, sodium chloride, and sodium glutamate (Nacalai Tesque, Kyoto, Japan) were used as additives.

2.2. Preparation of silicone formulations

The formulations used in this study are listed in Table 1. Silicone elastomer Silastic® Q7-4750 is supplied as a two-component kit (parts A and B). Part A contains a platinum catalyst and part B contains a cross-linker which has silicone hydride groups (Si-H). This material is cross-linked after mixing together the two parts A and B, via an addition reaction of silicone hydride groups to silicone vinyl units assisted with the catalyst. HSA, IFN/HSA and IFN/HSA/additive powder were prepared using the procedure outlined in a previous paper [3]. The preparation method of a matrix-type formulation is also described in that paper. Typically, a covered-rod-type formulation C-1 was prepared as follows. Silastic® Q7-4750 part A (4.02 g) was mixed with part B (4.02 g) and IFN/HSA/additive powder (3.96 g). The IFN/HSA/additive powder content was 33% (w/w). A kneaded mixture of the IFN/HSA/additive powder and silicone was filled into a syringe. Separately, a mixture of part A (25 g) and part B (25 g) was filled into an another syringe. The mixtures were extruded through a concentrically arranged die (opening diameter was 1.9 mm) so that the IFN/HSA/additives containing silicone formed the inner part and the IFN/HSA/additive-free silicone the outer part. The extruded rod was cured at room temperature for 3 days. After curing, the rod was cut to 1 cm in length and stored at 5°C. The

Table 1
Composition of silicone formulations

	IFN (IU)	Composition		Powder size (μm)	Formulation type
		HSA (%)	Additive (%)		
M-1	–	30	–	53–150	Matrix
M-2	6×10^6	30	–	53–150	Matrix
M-3	–	30[a]	Glycine 10%	<2	Matrix
C-1	9×10^6	30	Glycine 2%, NaCl 1%	<2	Covered-rod
C-2	–	30	–	53–150	Covered-rod
C-3	1×10^6	20	–	<20	Covered-rod
C-4	1×10^6	30	–	<20	Covered-rod
C-5	1×10^6	40	–	<20	Covered-rod
C-6	1×10^6	50	–	<20	Covered-rod
C-7	1×10^6	30	–	<20	Covered-rod
C-8	1×10^6	30	–	20–53	Covered-rod
C-9	1×10^6	30	–	53–150	Covered-rod
C-10	1×10^7	30	Glycine 10%	<2	Covered-rod
C-11	1×10^7	30	Sodium glutamate 10%	<2	Covered-rod
C-12	1×10^7	30	NaCl 10%	<2	Covered-rod
C-13	1×10^7	30	–	<2	Covered-rod
C-14	–	30[a]	Glycine 10%	<2	Covered-rod

[a] Texas-Red-labeled HSA.

mixing ratio of IFN, HSA and additive solutions, or that of powder and silicone was changed according to the composition of each sample.

Texas-Red-labeled HSA was used to prepare M-3 and C-14 so that HSA could be identified by confocal laser scanning microscopy (CLSM). The labeling method has been reported by Titus et al. [4].

2.3. Measurement of IFN content in the silicone formulations

The IFN covered-rod-type formulation C-1 was frozen with liquid nitrogen and then milled for 30 s using a Cryo-Press CP-100 (Microtech, Chiba, Japan). The milled pieces were immersed in 30 ml of phosphate-buffered saline (PBS, pH 7.4), 0.3% Tween 20 and 0.01% sodium azide. The solution was diluted with PBS containing 0.5% bovine serum albumin (BSA) and assayed for IFN concentration by bioassay at Mitsubishi Kagaku BCL (Tokyo, Japan).

2.4. In vitro release study

An IFN silicone formulation (C-3–13) was placed in a tube containing 10 ml of PBS (pH 7.4), 0.5% HSA and 0.01% sodium azide. Since IFN is unstable at 37°C in a solution form, the tube was placed in an incubator at 5°C to avoid degradation of IFN and to measure the precise amount of IFN released. At designated times, an aliquot was collected and transferred to a new tube containing fresh buffer. The solution was assayed for IFN concentration by radioimmunoassay (RIA), using an Interferon-α kit (Dinabot, Tokyo, Japan). RIA was performed in duplicate. IFN is stable in the buffer during the sampling intervals.

In vitro release study of HSA silicone formulation (M-1, C-2) was performed in the same manner except that the release medium was 3 ml of PBS (pH 7.4) and 0.01% sodium azide, and that HSA concentration was assayed by the Bradford method.

2.5. Release rate analysis

The release rate was analyzed based on the in vitro release profile. It was calculated by the least-squares method.

2.6. Measurement of osmotic pressure

IFN/HSA or IFN/HSA/additive powder (300 mg) was dissolved in 0.6 ml of PBS (pH 7.4), 0.5% BSA and 0.01% sodium azide. The osmotic pressure of the solution was measured three times, using an auto-osmometer (Osmostat OM-6020, Daiichi Kagaku), for each sample.

2.7. Experimental animals

Female athymic nude mice (nu/nu, BALB/c background) were purchased from CLEA Japan (Tokyo), and were housed in a negative-pressure safety rack under specific pathogen-free conditions.

2.8. Measurement of serum IFN levels

The IFN covered-rod-type formulation C-1 was administered subcutaneously into 5-week-old mice. Then, blood samples were collected at preset time points of 1, 4, 7, 14 and 28 days. Sera were collected and stored at −40°C until the IFN assay. The serum IFN levels were measured by RIA, using an interferon-α kit.

2.9. Pharmacokinetic analysis

The apparent half-life ($T_{1/2}$) was calculated using a one-compartment model as follows. The elimination rate (K_e) was calculated by the least-squares method from the linear terminal phase of the serum concentration–time curve and used to calculate $T_{1/2}$ from the following relationship: $T_{1/2}=\ln 2/K_e$.

2.10. Confocal laser microscope analysis

Silicone formulations M-3 and C-14 were prepared with Silastic® Q7-4750 silicone and Texas-Red-labeled HSA/glycine powder (powder size, <2 μm; Texas-Red-labeled HSA content, 30% (w/w); glycine content, 10% (w/w)). The silicone formulation was placed in a tube containing 10 ml of PBS (pH 7.4), 0.5% BSA. The tube was placed in an incubator at 5°C for 5 days. The initial and in vitro tested formulations were cut in the center and observed using a CLSM (LSM-GB200, Olympus Optical). HSA was rendered fluorescent by Texas-

Red, while silicone elastomer was not. Therefore, the HSA powders distributed in silicone elastomer could be detected against the black background of silicone elastomer.

3. Results

3.1. Preparation of covered-rod-type formulation

HSA was used as a model protein drug. IFN was used as an example of a protein drug which exerts physiological activities at very low concentrations. The preparation method of a covered-rod-type formulation is simple and the reproducibility of the formulation is good. The thickness of the outer layer was uniform and there was no space between the outer and the inner layers. Fig. 1 shows an example of a covered-rod-type formulation.

In order to evaluate the stability of IFN during the preparation of the covered-rod-type formulation, the content of IFN in the formulation was determined. The IFN covered-rod-type formulation C-1 was crushed using a grinder. The pieces were then immersed in buffer, and the resultant solution was bioassayed for IFN content (Table 2). The recovery of IFN from the IFN covered-rod-type formulation was approximately 100%, indicating that IFN was stable during the preparation of the covered-rod-type formulation.

3.2. In vitro release behavior

HSA was continuously released from the matrix-type formulation M-1 over a period of approximately 1 month (Fig. 2a). The release rate was high during the initial stage but decreased with time. On the contrary, the covered-rod-type formulation C-2 continued to release HSA at a constant rate; the total amount of HSA released in 99 days was 82% (Fig. 2b).

The profiles of HSA release of the matrix-type and covered-rode-type formulations are approximated by Eqs. (1) and (2), respectively. The squares of the correlation coefficients between the calculated and measured values are indicated in the figures. The HSA release profiles of the matrix-type and covered-rod-type formulations correspond very well to Eqs. (1) and (2), respectively.

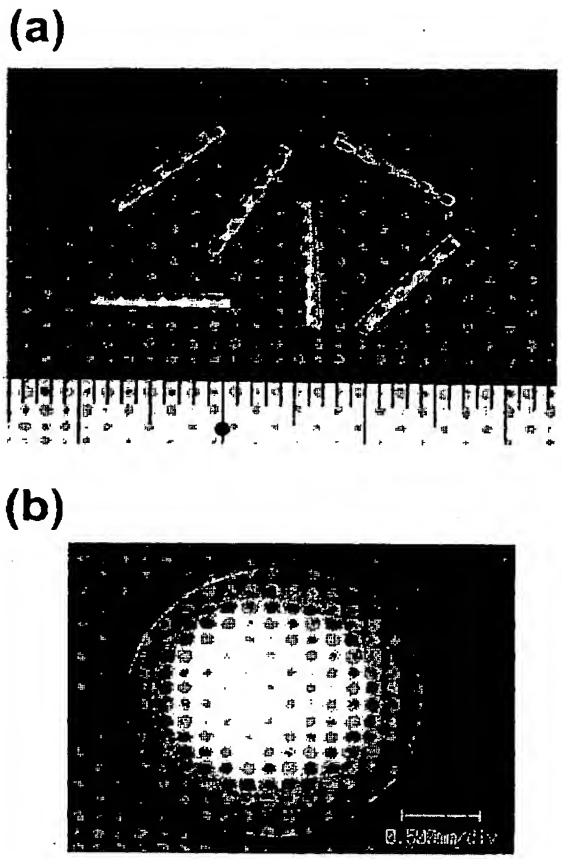

Fig. 1. Photographs of a covered-rod-type formulation: (a) the appearance, and (b) a cross-section.

$$y = a_m \cdot t^{1/2} + b_m \quad (1)$$

Table 2
Recovery of IFN from IFN covered-rod-type formulation[a]

Sample	IFN content	
	Theoretical value (IU)	Recovery (%)
C-1	9.25×10^6	102±6

[a] Results expressed as mean±deviation of two independent samples.

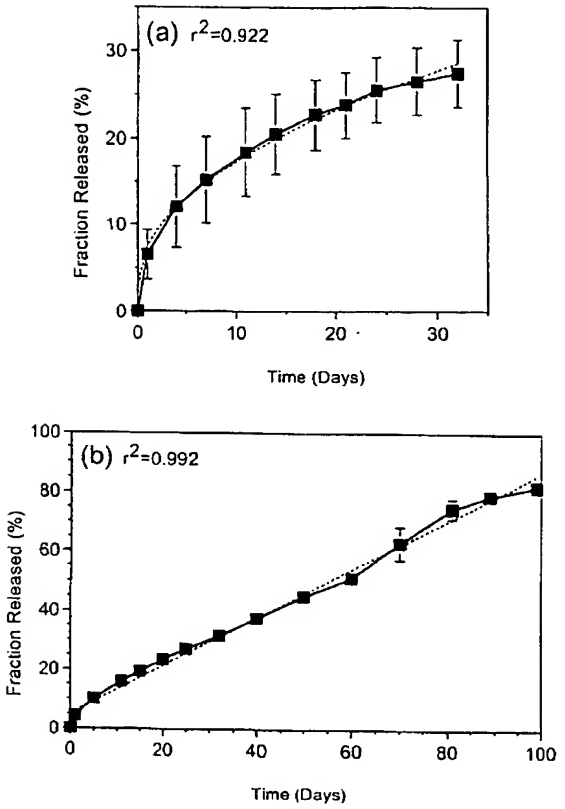

Fig. 2. HSA release profiles for HSA silicone formulations: (a) matrix-type, (b) covered-rod-type. Each point represents mean±deviation of data from two independent samples.

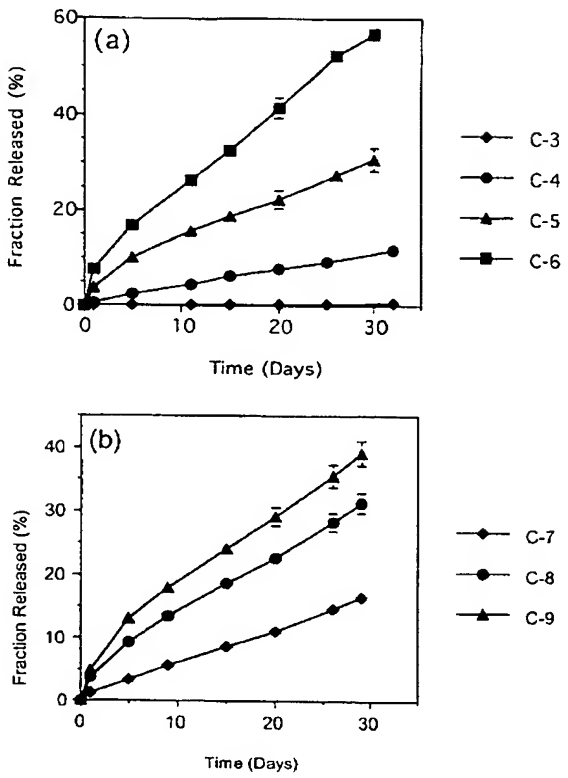

Fig. 3. IFN release profiles for IFN covered-rod-type formulations containing (a) different amounts of IFN/HSA powder, and (b) IFN/HSA powders of different particle sizes. Each point represents mean±deviation of data from two independent samples.

$$y = a_c \cdot t + b_c \quad (2)$$

3.3. Control of drug release

3.3.1. Effect of IFN/HSA powder content

Covered-rod-type formulations containing different amounts of IFN/HSA powder were prepared. These formulations were immersed in buffer to assay for IFN release. Fig. 3a shows the results.

The total amount of IFN released over 1 month for C-3 (IFN/HSA powder content, 20%) was extremely small, 0.65%; however, IFN was released continuously at a constant rate from C-4, C-5 and C-6, whose IFN/HSA powder contents were 30% or more. The IFN release rate increased with increasing IFN/HSA powder content. The total amount of IFN released over 1 month for C-6 (the highest content of IFN/HSA powder) was 57%.

3.3.2. Effect of IFN/HSA particle size

Covered-rod-type formulations containing IFN/HSA powders of different particle sizes, i.e., C-7 (<20 μm), C-8 (20–53 μm) and C-9 (53–150 μm), were immersed in buffer to assay for IFN release. Fig. 3b shows the results.

The IFN release rate increased with increasing particle size of IFN/HSA powder. The total amount of IFN released over 1 month for C-7 (smallest particle size) was 17%, whereas that for C-9 (largest particle size) was 39%.

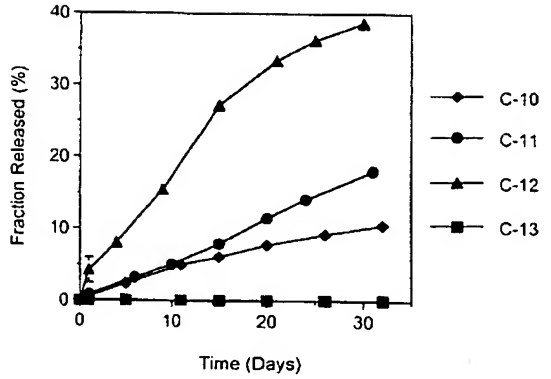

Fig. 4. IFN release profiles for IFN covered-rod-type formulations containing different additives. Each point represents mean±deviation of data from two independent samples.

3.3.3. *Effect of kinds of additives*

Covered-rod-type formulations were prepared using IFN/HSA powder containing different types of additives: C-10 (additive: glycine), C-11 (additive: sodium glutamate), C-12 (additive: sodium chloride) and C-13 (no additive). The formulations were immersed in buffer to assay for IFN release. Fig. 4 shows the results. The IFN release rate depends on the kinds and presence/absence of additives.

IFN/HSA/additive or IFN/HSA powders used to prepare C-10–C-13 were dissolved in a buffer, and the osmotic pressures of the buffers were measured. Table 3 summarizes the IFN release rates and differences in osmotic pressure (which is equal to the osmotic pressure of the buffer containing IFN/HSA/additive minus the osmotic pressure of the buffer itself). The osmotic pressure of the buffers in which IFN/HSA/additive powder (C-10–C-12) was dissolved was higher than that without additive (C-13).

Table 3
IFN release rates of IFN covered-rod-type formulations and osmotic pressure of solutions with dissolved IFN/HSA/additive powder[a]

	Release rate, a_c (%/day)	Osmotic pressure (mOsm)
C-10	0.335±0.061	1468±2
C-11	0.586±0.040	1761±8
C-12	1.329±0.003	4841±5
C-13	0.001±0.000	148±3

[a] Results of release rates expressed as mean±deviation of two independent samples. Results of osmotic pressures expressed as mean±S.D. of three measurements.

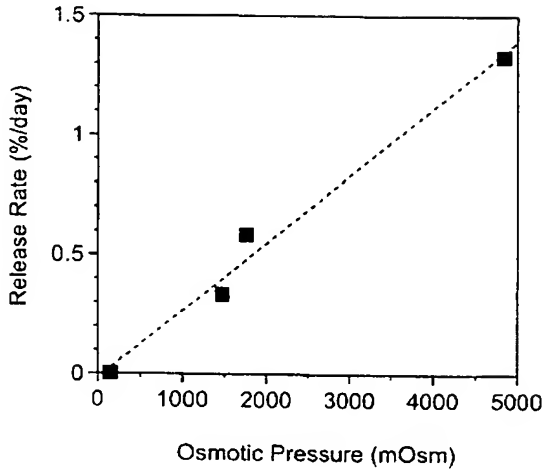

Fig. 5. Correlation between IFN release rates and osmotic pressure.

The relationship between IFN release rate (corresponding to a_c in Eq. (2)) and the osmotic pressure is shown in Fig. 5. The IFN release rate increased with increase in the osmotic pressure.

3.4. *Effect of length of the covered-rod-type formulation*

Covered-rod-type formulations C-8 of different lengths (1 and 2 cm) were immersed in buffer to assay for IFN release. Fig. 6 shows the change in IFN released per day with time.

Over the first month, both formulations released IFN at a constant rate, except on the first day; the amount of IFN released per day from 1- and 2-cm formulations were similar. However, after 1 month, the amount of IFN released from the 1-cm formulation declined gradually, whereas that released from the 2-cm formulation remained constant over 2 months.

3.5. *Change in serum IFN concentrations*

The IFN covered-rod-type formulation C-1 was

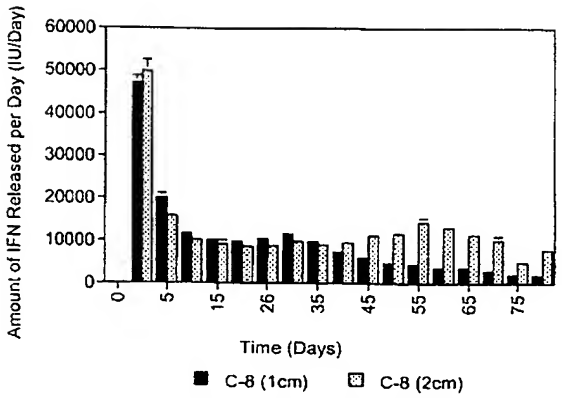

Fig. 6. The amount of IFN released per day from IFN covered-rod-type formulations with different rod lengths. Each point represents mean±deviation of data from two independent samples.

administered subcutaneously into the back of nude mice. Blood samples were collected from the nude mice at predetermined time points after administration, and the serum IFN concentrations were measured. Fig. 7 and Table 4 show the changes in serum IFN concentrations and the half-lives, respectively. For reference, the data for the IFN matrix-type formulation M-2 and IFN aqueous solution previously reported are also shown in Fig. 7 and Table 4.

In the group of mice in which IFN aqueous

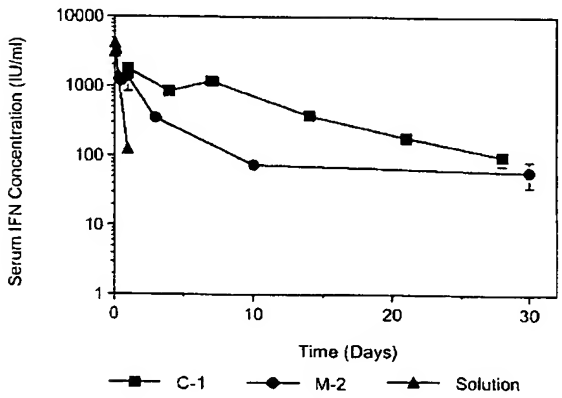

Fig. 7. The time course of serum IFN levels in nude mice after administration of IFN silicone formulations. Each point represents mean±S.D. of data from three nude mice. (■) C-1; (●) M-2; (▲) solution.

Table 4
Pharmacokinetic parameters

Parameter		C-1	M-3[3]	Solution [15]
Dose	(IU)	9×10^6	6×10^6	1×10^5
$T_{1/2}$	(h)	158	190	4

solution (1×10^5 IU) was administered, the serum IFN concentration decreased rapidly. In the group of mice in which the IFN matrix-type formulation M-2 (6×10^6 IU) was implanted, the maximum serum concentration of IFN (1379±657 IU/ml) was observed at the initial sampling time, i.e., 24 h after implantation; the concentration decreased to approximately 1/19 of the maximum value at 10 days after implantation; subsequently, serum IFN could be detected for 30 days. On the other hand, in the group of mice in which the IFN covered-rod-type formulation C-1 (9×10^6 IU) was implanted, the maximum serum concentration (1763±137 IU/ml) was observed at 24 h, and an approximately constant level was maintained; even on the 28th day after implantation, the serum IFN concentration was approximately 1/18 of the maximum value.

3.6. Mechanism of drug release

The change in distribution of Texas-Red-labeled-HSA powder in silicone with time was analyzed by CLSM. Fig. 8 shows micrographs of the formulations.

Before immersion of the formulations in buffer, Texas-Red-labeled-HSA powder was homogeneously distributed throughout silicone; there was no difference in the distribution of Texas-Red-labeled-HSA powder between the matrix-type formulation M-3 (Fig. 8a), and the inner layer of the covered-rod-type formulation C-14 (Fig. 8b). After immersion for 5 days, Texas-Red-labeled-HSA powder was released, leading to a decrease in the density of the powder in M-3 (Fig. 8c). On the other hand, in C-14, water infiltrated from the ends of the formulation, and the area where powder was released became transparent. At the ends of the formulation, little powder was present; in contrast, as much powder as under the initial condition remained in the central part of the formulation (Fig. 8d).

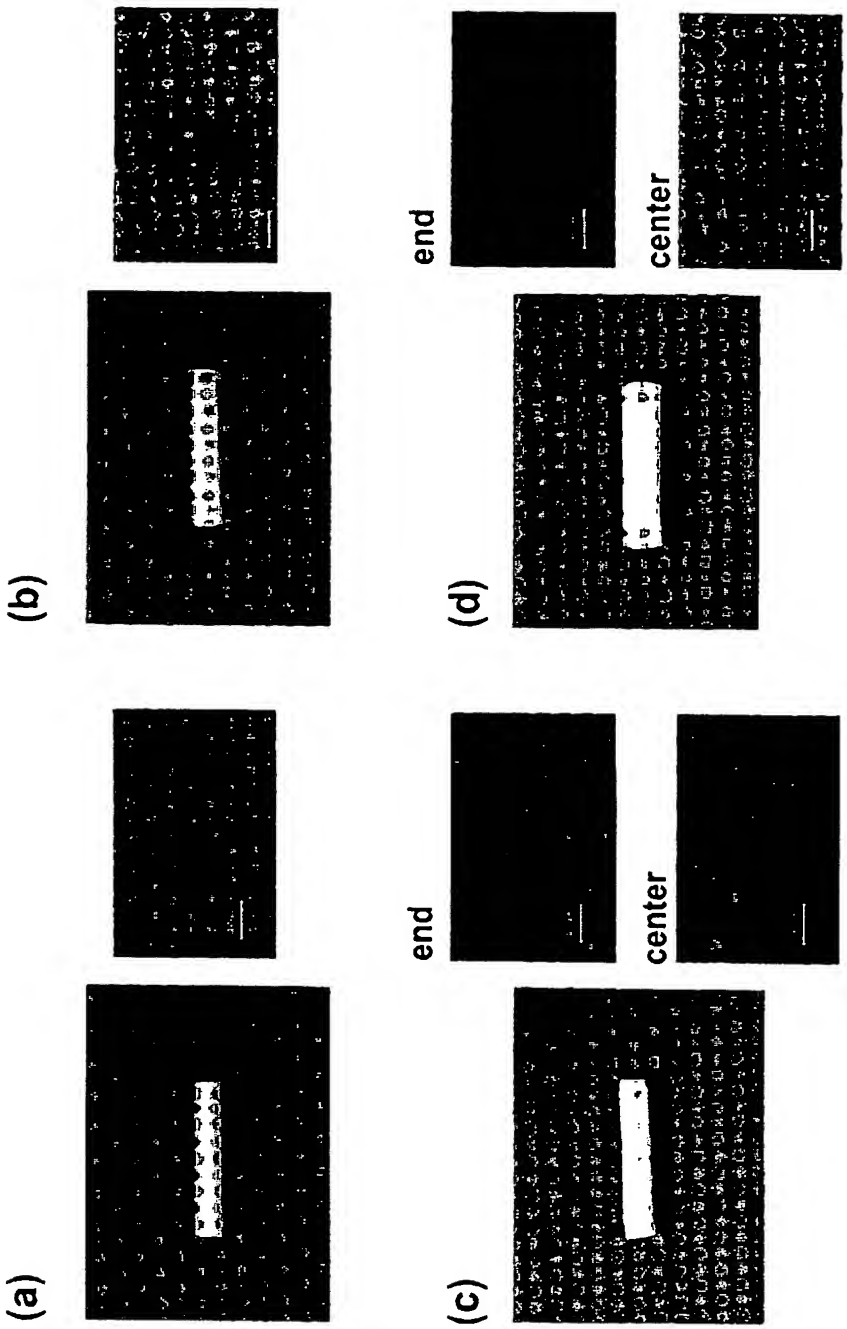

Fig. 8. Time-course images of the silicone formulations. (a) Before release test, M-3. (b) Before release test, C-14. (c) Incubated for 5 days in vitro, M-3. (d) Incubated for 5 days in vitro, C-14. Bars in CLSM images indicate 10 μm.

4. Discussion

In general, peptide and protein drugs are unstable and have an extremely short half-life in the body. To resolve these clinical problems, many researchers have studied sustained-release formulations of protein drugs in order to increase the duration of the drug effect and reduce the frequency of administration and side effects. Examples of formulations which have already been practically applied include microspheres of poly(lactic/glycolic acid) such as Lupron Depot® [5–8] and Nutropin Depot™ [9–13]. These microspheres of poly(lactic/glycolic acid) release drugs via an initial burst immediately after administration. It is reported that the initial burst of poly(lactic/glycolic acid) microspheres is attributed to their large surface area and/or porous structure [7,10,11]. Due to the initial burst, microspheres of poly(lactic/glycolic acid) can be used as carriers of drugs with specific pharmacological effects (e.g., leuprorelin acetate) and those with a wide therapeutic index (e.g., growth hormone); however, an initial burst is generally undesirable from the point of view of safety.

In order to develop a sustained-release formulation of protein drugs that is effective over a long time of period, we have developed a matrix-type formulation using silicone as a carrier. The matrix-type formulation was designed to suppress initial burst, by reducing surface area compared with microspheres, and making the structure not porous. Although the level of the initial burst is not as high as that of microsphere formulations, the release rate of the protein drugs decreases with time. Based on the knowledge accumulated through the development of matrix-type formulations, we have improved the geometry of formulations and succeeded in developing a new formulation, i.e., a covered-rod-type formulation. Since the covered-rod-type formulation was designed to further reduce surface area compared with the matrix-type formulation, initial rapid release of the covered-rod-type formulation is very little. In addition, the covered-rod-type formulation enables the release of protein drugs at a constant rate over a long period.

The protein drug release profiles from matrix-type and covered-rod-type formulations were examined using HSA (Fig. 2). The amount of HSA released from the matrix-type formulation M-1 decreased with time, while the covered-rod-type formulation C-2 continued to release HSA at a constant rate for approximately 100 days. It is reported that when a drug dispersed in polymer is released through channels, the percentage of drug released is proportional to the square root of time [14]; $y = a_m \cdot t^{1/2}$. On the other hand, when a drug is released at a constant rate regardless of time (zero-order release), the percentage of drug released is in proportion to time; $y = a_c \cdot t$. Theoretically these equations intersect the origin. However, since protein drug particles which exist on the surface of formulations are released immediately after immersion into an aqueous environment, coefficients b_m and b_c were necessary in the equations to make corrections for an initial rapid release (Eqs. (1) and (2)). The HSA release profile of the matrix- and covered-rod-type formulations, M-1 and C-2, corresponded very well to Eqs. (1) and (2), respectively.

When covered-rod-type formulations containing IFN/HSA powder were immersed in buffer, IFN was released continuously in the zero-order manner. The IFN release rate depended on the IFN/HSA powder content and its particle size. In addition, when covered-rod-type formulations were prepared using IFN/HSA powder containing different types of additives, e.g., glycine, sodium glutamate and sodium chloride, the IFN release rate was influenced by the kind of additive (Fig. 4). The degree of influence was greater for formulations prepared using an additive that induces a higher osmotic pressure (Table 3 and Fig. 5). Previously, we performed similar experiments using matrix-type formulations and reported the results [3]. It was shown that powder content, particle size and additives had similar effects on the protein drug release rates of these two formulations; however, the release profiles of the matrix-type formulations were of the first order, while those of the covered-rod-type formulations were of zero order.

We measured the serum IFN concentrations of nude mice in which covered-rod-type formulations were implanted. As a comparison, in the group of mice in which IFN solution was administered, the serum IFN concentration decreased rapidly. In the group of mice in which the IFN matrix-type formulation M-2 or IFN covered-rod-type formulation C-1

was implanted, the serum IFN concentration was maintained above the detection limit during the period of observation, i.e., about 30 days after a single administration (Fig. 7). The half-lives ($T_{1/2}$) were 48 and 40 times longer than that of an IFN aqueous solution, respectively (Table 4). This indicates that the both formulations released IFN continuously in vivo. In the group of mice in which the IFN covered-rod-type formulation was implanted, the serum IFN concentration was maintained at an approximately constant level for 28 days, indicating that IFN was released from the covered-rod-type formulation at a constant rate not only in vitro but also in vivo.

Microspheres are difficult to apply for protein drugs such as cytokine and insulin that manifest strong physiological activity at low doses, because of a large initial burst. However, it is expected that the covered-rod-type formulation can be practically applied for these protein drugs.

The release mechanism of protein drug from a covered-rod-type formulation is discussed with respect to the formation process of channels, water infiltration patterns and diffusion characteristics of the protein drug, by comparing these aspects with those of a matrix-type formulation, as follows.

The release process of protein drugs from a matrix-type formulation is considered to be as follows. First, protein drug particles existing near the surface of the formulation are dissolved and released into the surrounding water. Second, adjacent particles are dissolved and released into the surrounding water. When high osmotic pressure is induced by the dissolution of protein drug powder into water, cracks occur on the surrounding silicone walls, which promotes the connection of the channels. By repeating this process, pores (where the protein drug powder had been) and cracks connect to form channels, and the protein drug inside the formulation is sequentially released. In case of a covered-rod-type formulation, protein drugs are considered to be released through channels which are formed in an inner layer by the same process as the matrix-type formulation. In fact, the release rates of protein drugs can be controlled by the same factors such as the content of protein drug powder, the particle size, and the kind of additives, with respect to both the matrix- and covered-rod-type formulations as described above. The results indicate that the release processes of the two formulations are related to channel formation. Protein drugs are not released through an outer layer of the covered-rod-type formulation, because channels are not formed in the outer layer and protein drugs cannot diffuse through polymer network.

In order to investigate the water infiltration patterns of the matrix- and the covered-rod-type formulations, Texas-Red-labeled HSA powders were used to prepare both formulations. The change in the distribution of Texas-Red-labeled HSA powder within the formulations was analyzed by CLSM, tracing the fluorescence of Texas-Red (Fig. 8). Before the immersion of the formulations in buffer, the images of the matrix-type formulation and of the inner layer of the covered-rod-type formulation were similar; Texas-Red-labeled HSA powders were homogeneously distributed throughout silicone. For the matrix-type formulation incubated for 5 days in vitro, the image of the central part is similar to that of the two ends; Texas-Red-labeled HSA powder was released and its density decreased. On the other hand, for the covered-rod-type formulation incubated for 5 days in vitro, the image of the central part is different from that of the ends. In the central part, Texas-Red-labeled HSA powder existed at a density similar to the initial density; it is considered that the release of Texas-Red-labeled HSA does not occur from the central part at that time. In contrast, at the two ends, only a slight amount of dissolved Texas-Red-labeled HSA remained. This may be explained as follows. In the matrix-type formulation, water infiltration proceeded on the entire surface of the formulation; water infiltrated into all parts of the formulation within a relatively short period, and therefore a similar image was observed for all parts of the formulation at day 5. In contrast, in the covered-rod-type formulation, water infiltration proceeded from the ends of the formulation, the rate of water infiltration was slow and at the central part the initial condition was maintained at day 5.

The water infiltration patterns of matrix-type and covered-rod-type formulations are summarized as follows. For a matrix-type formulation, the formation of channels occurs over the entire surface of the formulation; since the water penetration front proceeds from the surface of the cylindrical formula-

tion into its interior, the area of the water penetration front decreases with time (Fig. 9a). For a covered-rod-type formulation, since the formulation is surrounded by silicone through which water does not penetrate, the area from which water can infiltrate is limited to the two ends; the area of the water penetration front always remains constant (Fig. 9b). The above discussions are also supported by the observation obtained in the in vitro experiments, that water infiltrates throughout the matrix-type formulation, but water infiltrates only from the two ends of the covered-rod-type formulation (Fig. 8).

Protein drug inside a covered-rod-type formulation is dissolved upon water infiltration. However, the dissolution of the protein drug is not the rate-limiting step of drug release, because the release of protein drug from a covered-rod-type formulation continues even after water infiltrates into all parts of the formulation, as evident by visual observation. In addition, if the dissolution of protein drug powder is the rate-limiting step of the drug release, the release rate should decrease with time, since the dissolved protein drug must diffuse through a longer channel as the water penetration front of a covered-rod-type formulation moves inward with time. However, the results were inconsistent with this assumption; a covered-rod-type formulation continued to release protein drug for a long period at a constant rate. These findings indicate that the diffusion rate of the dissolved protein drug inside the formulation is extremely low compared to that outside the formulation.

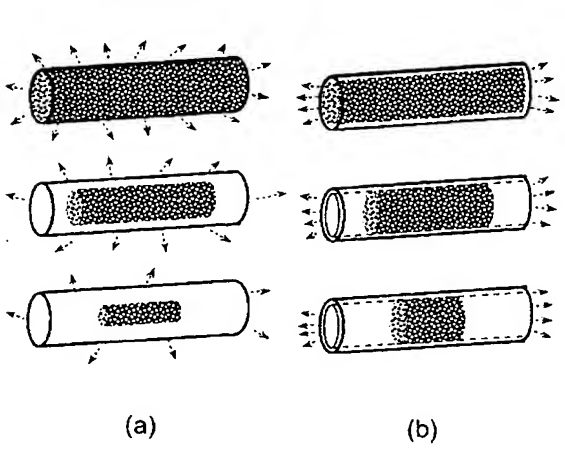

Fig. 9. Images of water penetration into and protein release from the silicone formulations: (a) matrix-type, (b) covered-rod-type. The patterned domain indicates the area where intact protein drug powders remain. Arrows indicate from where the protein drug is released.

In the case of a matrix-type formulation, protein drug is released from the entire surface of the formulation; the concentration of the protein drug inside the formulation decreases within a relatively short period. In contrast, diffusion of protein drug is suppressed in a covered-rod-type formulation, since (1) the area from which the drug is released is extremely small compared to that of the matrix-type formulation and (2) the channels are rendered narrow by the pressure of the outer layer on the inner layer. Consequently, the concentration of the dissolved protein drug inside the channels might be saturated and remains constant, resulting in the constant release of the protein drug.

The mechanism explained above is also supported by the finding that the release duration can be controlled by the length of the formulation. When 1- and 2-cm covered-rod-type formulations C-8 were immersed in buffer to assay for the amount of IFN released per day, the amounts of IFN released from the two formulations were similar over the first month; however, subsequently, the IFN released from the 1-cm formulation gradually decreased, whereas that from the 2-cm formulation was maintained at the same level as during the first month (Fig. 6). Over the first month, there was no remaining IFN/HSA powder to be dissolved inside the 1-cm formulation and the IFN concentration inside the channels gradually decreased, as did the release rate. In contrast, the IFN concentration inside the channels was still maintained at a constant level inside the 2-cm formulation, which leads to a continuous zero-order release.

The coefficient a_m in Eq. (1) is considered to be dependent on the drug release area, the solubility of the protein drug in water (body fluid), the channel volume inside the formulation, the tortuosity of the channels and the diffusion rate of the dissolved protein drug inside the channels. The coefficient a_c in Eq. (2) seems to be influenced by the same factors as those of a_m in Eq. (1); however, even if the matrix-type formulation and the inner layer of the

covered-rod-type formulation have the same composition, the values of a_c and a_m would not be the same. It is considered that the difference between a_c and a_m depends not only on the difference between the drug release area but also on other factors, such as the diffusion rate of the dissolved protein drugs in the channels, as discussed above. For example, a_m of the matrix formulation D-4 was 16.3%/day, as reported in the previous report [3], whereas a_c of the covered-rod-type formulation C-10, having an inner layer of the same structure as that of D-4, was 0.335%/day. Even if the coefficients a_m and a_c are corrected by the difference in the drug release area (the drug release area of D-4 is approximately 14 times that of C-10), the coefficient for the matrix-type formulation is higher than that for the covered-rod-type formulation. This may be because the IFN diffusion rate inside the channels of the covered-rod-type formulation is lower than that of the matrix-type formulation.

In general, protein drugs including IFN are denatured and deactivated by treatments such as heating or the use of organic solvents. Since the covered-rod-type formulation can be prepared under mild conditions without heat treatment or the use of organic solvents, it is possible to prepare formulations of protein drugs that are sensitive to these treatments without reducing the physiological activity. In fact, the evaluation of the stability of IFN during the preparation of the formulation revealed that the recovery of IFN from the formulation was approximately 100% of the theoretical value (Table 2).

5. Conclusion

In order to develop a long-term delivery system for protein drugs that enables the zero-order release, a covered-rod-type formulation was developed.

Since a covered-rod-type formulation can be prepared under mild conditions without heat treatment or the use of organic solvents, it can be applied to protein drugs that are sensitive to these treatments which generally cause degradation of their physiological activities. Furthermore, the covered-rod-type formulation can release protein drug at a constant rate regardless of the molecular weight of the drug without significant initial burst. The drug release rate can be controlled by changing parameters such as the particle size of protein drug powder, its content and the kind of additives. When the IFN covered-rod-type formulation was implanted in vivo, the serum IFN concentration was maintained at a constant level.

The covered-rod-type formulation is expected to be practically applied for protein drugs with narrow therapeutic indices, and can be used effectively and safely for treatments of chronic diseases.

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